Immune Function, Body Composition and Genetic Correlates of Bat 'White-Nose Syndrome'

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Overview

The main goals of this project are to describe possible differences in immune function as they relate to body composition in WNS affected and unaffected bats. We will also determine if body composition, especially amount of total body fat in little brown myotis (*Myotis lucifugus*), is associated with the likelihood of bats to prematurely emerge from hibernation. In addition, we are testing the capacity of bats to mount innate and adaptive immune responses against *G. destructans*, identify major histocompatibility complex (MHC) alleles associated with potential cell-mediated responses, and characterize MHC diversity in affected and unaffected bats. We will test the following four hypotheses:

H_1. There exists a threshold of fat reserves during hibernation below which little brown myotis cannot mount a sufficient immune response to resist invasion by pathogens.
H_2. Below this threshold in fat reserves, bats will begin to exhibit atypical behaviors for hibernation as they respond to distress associated with compromised immune function.
H_3. Little brown myotis are incapable of mounting a sufficient immune response to *Geomyces destructans*.
H_4. There is a genetic correlation between immunocompetence and survival after exposure to white-nose syndrome, which is evident in allelic composition at the MHC.

Progress To Date

Relationships Between Body Condition and Immune Response

We have completed body composition analysis on 167 little brown bats from affected and unaffected hibernacula through fall 2008 and winter 2009. These analyses will serve to establish general colony-wide levels of total body fat (TBF) and other metrics of body composition to be compared to body composition of bats sampled for immune function analyses. Samples represent affected sites: Aeolus Cave, VT, Chester mine, MA, Graphite Mine, NY, and Hibernia, NJ; and unaffected sites: Saltpeter Mine, KY, US Steel Mine, PA, CS&M Mine, PA, and North Ledges, OH. Using these samples, we compared total body fat (TBF; g) between affected and unaffected sites for adult bats during early hibernation in November 2008. Both females ($t=-6.50$, d.f.=58, $p<0.001$) and males ($t=-3.62$, d.f.=27, $p=0.001$) had significantly lower TBF at affected sites than at unaffected sites (Fig .1).

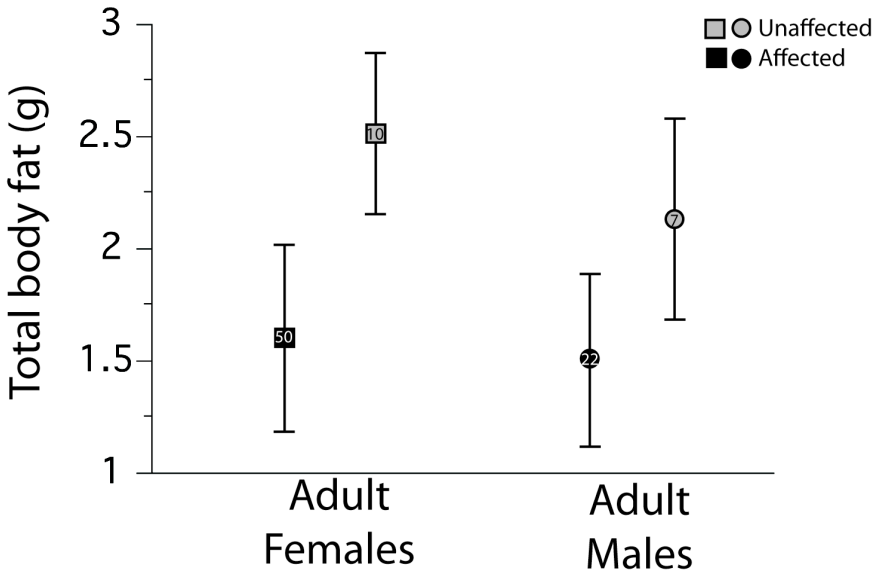

Figure 1. Total body fat (g) of adult female (squares) and male (circles) little brown myotis at affected and unaffected hibernacula in November 2008. Symbols and bars represent mean and standard deviation. Adult females at affected and unaffected sites had TBF=1.60±0.06g and 2.52±0.13g, respectively. Adult males at affected and unaffected sites had TBF=1.51±0.38g and 2.13±0.45g, respectively.

We have completed analyses of body composition for adult males, and juvenile bats during early, mid- and late hibernation at Aeolus Cave, VT (Fig. 2). These cohorts exhibited steep declines in TBF between early and mid-hibernation, but smaller (for adult males) or insignificant (for young males and females) changes in TBF between mid- and late hibernation. Adult females sampled for immune function assays will be added to this analysis to explore differences between sex and age cohorts through hibernation. Destructive body composition analysis is ongoing. Furthermore, we have determined that Body Mass Index (BMI = body mass (g) / length of forearm (mm)) provides a reasonable estimate of TBF for all cohorts sampled throughout fall and winter (Fig. 3). Thus, early analyses correlating various aspects of immune function to BMI provide encouraging preliminary results for hypothesized relationships.

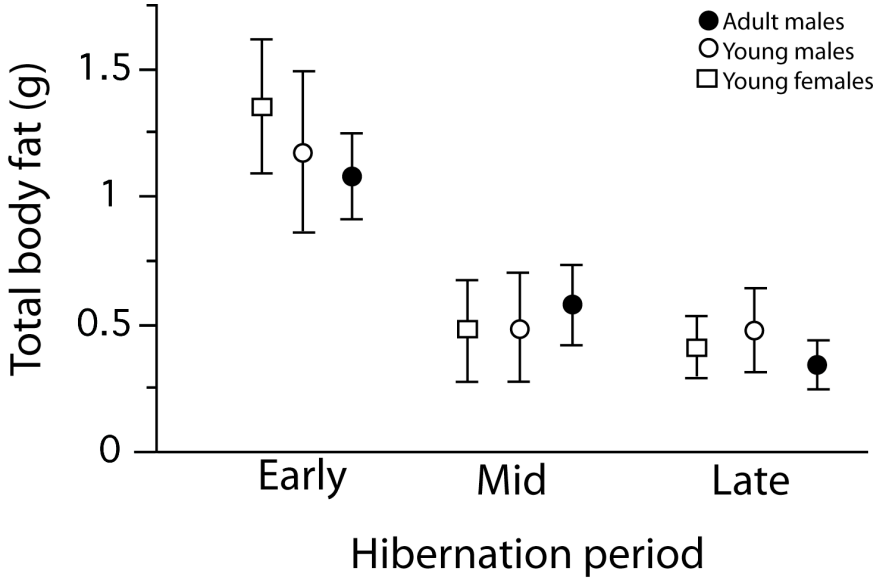

Figure 2. Total body fat (g) of young female (squares), young male (open circles), and adult male (closed circles) little brown myotis during early, mid- and late hibernation at Aeolus Cave, VT (affected). Symbols and bars represent mean and standard deviation. For both young females and young males, TBF was greatest in early hibernation, but did not differ significantly between mid- and late hibernation (Tukey-Kramer HSD test, α=0.05). For adult males, TBF was significantly lower with each successive hibernation stage (Tukey-Kramer HSD test, α=0.05). Within hibernation stages, TBF did not differ significantly among sex/age cohorts (ANOVA, p>0.05).

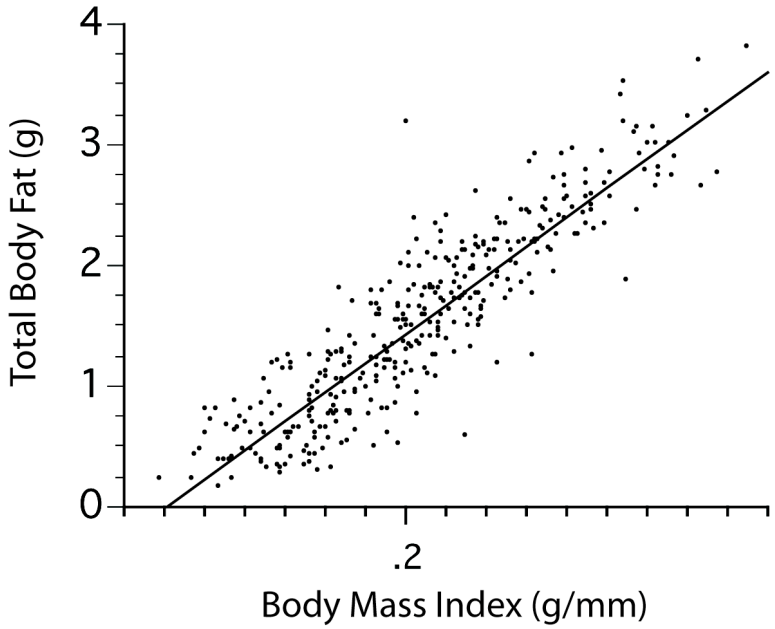

Figure 3. Relationship between body mass index (body mass (g)/length of forearm (mm)) and total body fat (g) for little brown myotis sampled during fall and winter at both affected and unaffected hibernacula (R^2=0.81, $F_{1,364}$=1542.7, p<0.001).

The Great Escape Hypothesis

During winter 2009, little brown myotis exhibiting atypical early emergence from hibernacula were colleted by New York State Department of Environmental Conservation and Vermont Fish and Wildlife Department. These samples are frozen and will soon be analyzed.

Immune Responses Against Geomyces destructans

During late summer 2009, we collected 12 blood samples from adult female little brown myotis to test the ability of this species to lyse *G. destructans* conidia using complement protein activity, an aspect of the constitutive innate immune response. Six of these samples were collected on 23 August 2009 and six were collected on 5 September 2009. These collections provided us with a preliminary representative sample from post-lactation, which from previous studies has shown to be the reproductive stage when complement protein activity is highest (compared to pregnancy and lactation; Moore et al., *in prep*). Using this group of samples, we compared the ability of blood from each individual to lyse *G. destructans*, *E. coli*, *S. aureus* and *C. albicans*. Our results show that blood from little brown myotis was significantly less able to kill *G. destructans* compared to *E. coli* (p<0.001), *S. aureus* (p=0.001), and *C. albicans* (p=0.033; Figure 4).

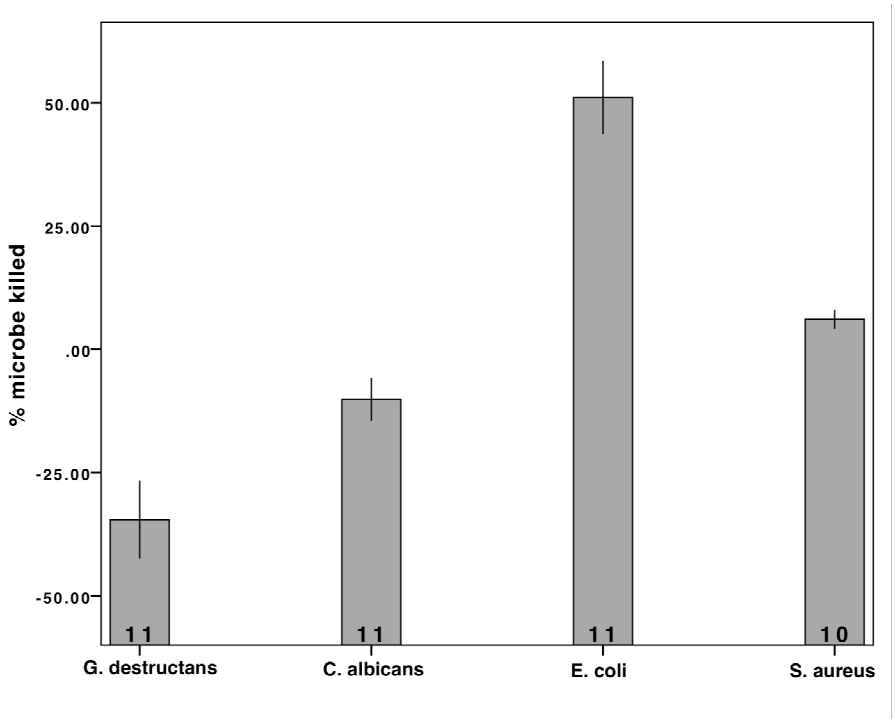

Figure 4. Bactericidal and fungicidal abilities against *G. destructans*, *C. albicans*, *E. coli*, and *S. aureus* in little brown myotis. Blood was collected from post-lactating little brown myotis during late summer 2009. Fungicidal ability against *G. destructans* (-34.55% ± 7.92) was significant lower compared to fungicidal ability against *C. albicans* (-10.18% ± 4.31; p=0.033), bactericidal ability against *E. coli* (51.09% ± 7.41; p<0.001), and bactericidal ability against *S. aureus* (6.10% ± 1.89; p=0.001). Bars indicate mean ± standard error. Numbers inside bars represent sample sizes.

During late hibernation 2010 (23 March 2010), we collected 18 blood samples from parous female little brown myotis hibernating in the unaffected Lawrence County Mine located in the Wayne National Forest in Nelsonville, OH. We assayed blood from these bats for fungicidal ability against *G. destructans*. In addition to testing the ability of hibernating bats unaffected by WNS to resist invasion by *G. destructans* at standard assay conditions (incubation of fungi/blood mixtures at ~21°C), we are testing for a possible relationship between fungicidal ability and body temperature. To do this, we incubated fungi/blood mixtures from each bats at 7, 14, 21, 28, and 35°C. We completed these assays during the week of 17 May 2010 and are currently incubating the prepared agar plates at 7°C. *G. destructans* colony forming units present on agar plates and used to calculate results will be ready to count in early June.

In addition to the *G. destructans* fungicidal assay, we are developing two methods to test immune responses specifically against the fungus. We have prepared for one of these assays, the lymphocyte proliferation assay, have all supplies on hand, and have run preliminary tests to see if a standard lymphocyte proliferation assay can be performed using non-lethal blood sampling in big brown bats (*Eptesicus fuscus*), which appear to be less susceptible to WNS and provide larger volumes of blood that can be more-safely collected from this species compared to *M. lucifugus*. Based on our initial results using whole blood, we will modify the assay to use white blood cells isolated from whole blood. We will test the modified form of this assay using fresh blood collected from *E. fuscus* starting in early June 2010.

Relationships Between Immune Responses Against G. destructans *and the MHC*

In addition to the 224 tissue samples (subsequent tissue samples are being collected from ALL bats in frozen storage for body composition analyses) that were collected during the 2008-2009

hibernation period for our research on the makeup and diversity at the MHC, we collected a total of 54 tissue samples from hibernating little brown myotis captured in Akron Mine, NY, Barton Hill Mine, NY, and Lawrence County Mine, OH. We will continue to collect samples from adult little brown myotis captured this summer within the affected area, which will represent individuals that likely survived at least one hibernation period exposed to *G. destructans*.

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